

# **Influence of Counterions on the Radius of Gyration of Phenylalanine Specific Transfer RNA as Determined by Small Angle X-Ray Studies**

**Ingrid PILZ and Otto KRATKY**

**Institut für Physikalische Chemie, Universität Graz**

**Friedrich VON DER HAAR and Friedrich CRAMER**

**Max-Planck-Institut für Experimentelle Medizin, Abteilung Chemie, Göttingen**

The influence of the ion and water cloud shell on the structure of polyelectrolytes is very important especially for small angle studies. In the present work the change in the radius of gyration of phenylalanine specific tRNA under the influence of various counterions was investigated. tRNA<sup>Phe</sup>(yeast) was studied in different solutions at pH 7.5, each containing only one certain kind of cation. As cations lithium, cesium, barium and spermidin were used. The influence of the ions on the radius of gyration of tRNA<sup>Phe</sup>(yeast) was considerable. The value of  $R$  was 23.6 Å with Li<sup>+</sup> ions, 26.2 Å with Cs<sup>+</sup> ions and 26.3 Å with Ba<sup>++</sup> ions; these results are discussed.

Primary structure of many tRNA species have been elucidated and the cloverleaf secondary structure by Holley [1] is generally adopted. Several detailed models for tertiary structure of tRNA molecules have been proposed [2–5]. From the coordinates of the atoms, the radius of gyration and the scattering curve of such models can be calculated and compared with the experimental data, as was done by Ninio *et al.* [3] and Connors *et al.* [4]. As a rule it is not absolutely necessary to use the coordinates of the single atoms [6] for the calculation and often the coordinates are assigned only to the scattering centers of base, sugar and phosphate groups. This approximation has no decisive influence on the calculated value of the radius of gyration or on the inner portion of the scattering curve.

The comparison of the experimental small angle X-ray data with those calculated for a particular model involves another problem. The experimental studies are carried out in solution and the influence of the solvent, the counterions and additional electrolytes on the radius of gyration is not well known.

Since the problem of the structure of the water shell could not be completely clarified even with simple ions [7], the theoretical treatment of the water shell and hydration presents very great difficulties with a polyelectrolyte such as tRNA.

Therefore the influence of the water and ion cloud shell is frequently ignored; some authors [3,4] assign one univalent ion to each phosphate group of the tRNA molecule for the calculation.

Theoretically, polyions have discrete charged groups, and for many calculations it is assumed that counterions are bound only to these discrete groups. In real polyions, however, a diffuse atmosphere of counterions in which the distribution of the counterions fluctuates must be assumed [7]. A theoretical treatment of this problem, based upon a geometric concept, was given by Luzzati *et al.* [8].

In order to compare the experimental data with the data calculated for the various suggested models it seemed important to discover more about the influence of both hydration and the binding of counterions. Consequently we investigated tRNA<sup>Phe</sup> in solution in several cationic forms.

Similar studies were effected by Luzzati *et al.* [8] and Bram [9] on DNA molecules; the authors determined the mass per unit length and the radius of gyration of the cross-section of DNA by using various counterions. The results obtained by the two groups are not in mutual agreement, as is discussed in detail by Eisenberg and Cohen [10].

## EXPERIMENTAL PROCEDURE

### MATERIALS

#### *Preparation of tRNA<sup>Phe</sup>(Yeast) with a Certain Cation*

77 mg of tRNA<sup>Phe</sup> from yeast prepared as described earlier [1] was dissolved in 20 ml of double-distilled water. 5 ml aliquots were dialyzed once against 0.4 M sodium phosphate pH 7.0, twice

against bidistilled water, once against 0.4 M sodium phosphate pH 7.0, twice against bidistilled water, three times against 0.1 N of univalent cation (0.1 N LiCl or 0.1 N CsCl), or three times against 0.01 N divalent cation (0.01 N BaCl<sub>2</sub> or 0.01 N spermidine chloride) solution and three times against double-distilled water to remove excess ions.

The dialyzed solution was freed of dust by passage along sintered glass and finally lyophilised.

#### *Solutions Used for the Small Angle Measurements*

The freeze-dried samples of tRNA<sup>Phe</sup>, each containing only one certain kind of cation, were dissolved in the following buffers: Li-tRNA in 0.05 M citric acid/LiOH pH 7.5, additional 0.1 M LiCl; Cs-tRNA in 0.05 M citric acid/CsOH pH 7.5, additional 0.1 M CsCl; Ba-tRNA in 0.05 M Tris, pH 7.5, in a second series 0.05 M BaCl<sub>2</sub> was added; spermidine-tRNA in 0.05 M Tris, pH 7.5, in a second series 0.05 M spermidine chloride was added.

#### METHODS

All small angle measurements were made with a highly stabilized X-ray generator using a copper tube at constant temperature (21°) and humidity of the room.

The camera as described elsewhere [11] and the step-scanning device [12,13] used have been described elsewhere. A proportional counter with pulse-height discriminator was used as a detector for CuK lines. Elimination of the CuK $\beta$  line was effected as described by Zipper [14]. A primary beam of line-shaped cross-section was used; the experimental curves therefore include the collimation effect: they are "slit-smeared". This is indicated in the figures by a tilde (e.g.  $\tilde{I}$ ,  $\tilde{R}$ ).

The collimation effect was eliminated according to the method of Guinier and Fournet [15] with the help of a computer program written in the Graz institute [16].

For the measurements, an entrance slit of 150  $\mu$  was used and the resolution corresponded to a Bragg spacing of 550 Å. All scattering curves were normalized to unit concentration, that is, the  $I/c$ -values were plotted.

#### RESULTS

With each type of tRNA<sup>Phe</sup> (Li-tRNA, Cs-tRNA, Ba-tRNA, spermidine-tRNA) several series were investigated, whereby the concentration of tRNA was varied from 2 mg/ml up to 20 mg/ml. The concentrations used are given in Table 1 for the series with different cations.

For better clarity the solutions investigated for each series are numbered according to the tRNA concentration. The Guinier plots [17] of the corresponding experimental scattering curves are numbered in the same way and shown in Fig.1 for Li-tRNA and in Fig.2 for Cs-tRNA. Fig.3 shows Ba-tRNA without additional barium salt and Fig.4 Ba-tRNA with the addition of 0.05 M BaCl<sub>2</sub>.

The statistical error is of course greater with solutions of low tRNA concentration and causes larger fluctuations of the measured intensity values. The curves of the solutions with higher concentrations of tRNA show deviations from the Guinier straight line caused by interference effects.

The apparent radii of gyration obtained from the slope of the slit-smeared curves shown in Figs.1—4 are also summarized in Table 1. (The scattering curves of spermidine tRNA showed a divergent behaviour. During X-ray irradiation, evolution of gas was observed and apparently some decomposition had occurred. Therefore a more detailed evaluation had to be abandoned.)

In Fig.5 the apparent radii of gyration ( $\tilde{R}$ ), summarized in Table 1, are plotted against the concentration of the corresponding tRNA<sup>Phe</sup> in solution. It can be clearly seen that the radius of gyration with Li<sup>+</sup> as cation is markedly smaller than with the

Table 1. Apparent radii of gyration ( $\tilde{R}$ ) of tRNA<sup>Phe</sup>(yeast) at various concentrations (c) of tRNA and with various counter ions

Li-tRNA			Cs-tRNA			Ba-tRNA			Ba-tRNA (+ BaCl <sub>2</sub> )		
Fig.1 Curve No.	c	$\tilde{R}$	Fig.2 Curve No.	c	$\tilde{R}$	Fig.3 Curve No.	c	$\tilde{R}$	Fig.4 Curve No.	c	$\tilde{R}$
	mg/ml	Å		mg/ml	Å		mg/ml	Å		mg/ml	Å
1	2.5	22.7	1	2.5	25.6	1	2.5	25.7	1	3.0	25.5
2	5.0	22.5	2	5.0	25.7	2	5.3	25.5	2	5.0	25.4
3	7.5	22.4	3	7.5	25.5	3	7.0	25.2	3	7.0	25.3
4	10.0	21.4	4	10	25.2	4	9.7	24.5	4	10	25.0
5	11.5	21.7	5	15	24.9	5	15.1	24.0	5	15	24.8
6	15	20.9	6	20	25.1	6	21.6	23.5	6	20	(26.3)
7	20	20.1									
	c → 0	23.2		c → 0	25.8		c → 0	25.9		c → 0	25.8

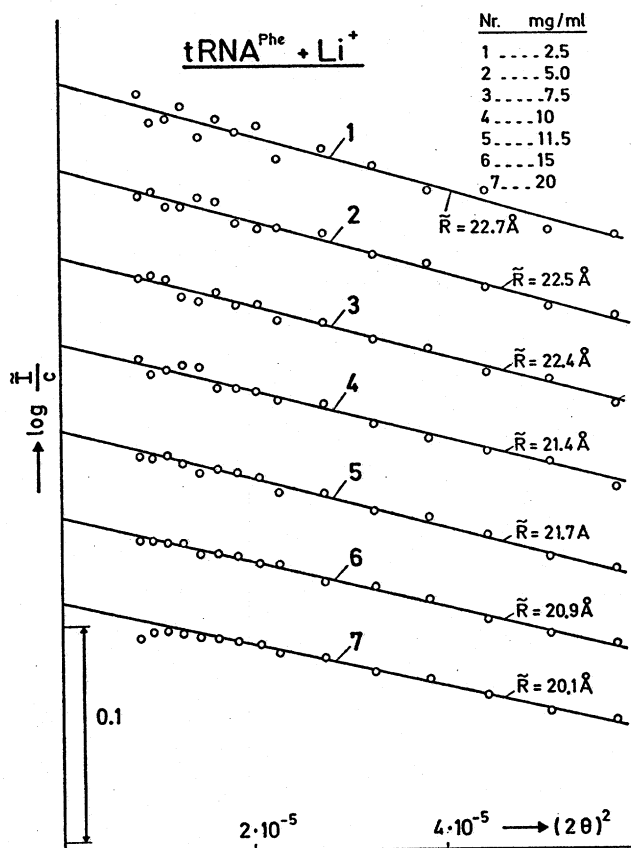


Fig. 1. Guinier plots of the scattering curves of  $\text{Li-tRNA}^{\text{Phe}}$  in 0.05 M citric acid/LiOH buffer, additional 0.1 M LiCl, pH 7.5. The concentrations  $c$  of  $\text{tRNA}^{\text{Phe}}$  and the apparent radii of gyration  $\bar{R}$  (obtained from the slope of the straight-lines) are also given in Table 1.  $2\theta$  is the scattering angle and  $\bar{I}$  the slit-smearred scattered intensity

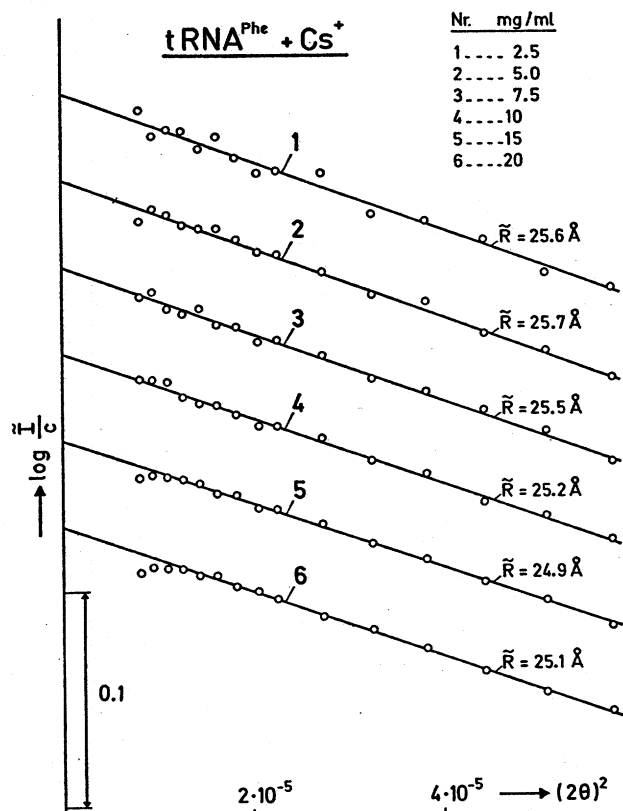


Fig. 2. Guinier plots of the scattering curves of  $\text{Cs-tRNA}^{\text{Phe}}$  in 0.05 M citric acid/CsOH buffer, additional 0.1 M CsCl, pH 7.5. The concentration of  $\text{tRNA}^{\text{Phe}}$  and the apparent radii of gyration are also given in Table 1

heavy cations  $\text{Cs}^+$  and  $\text{Ba}^{++}$ . Comparison of the scattering curves of  $\text{Ba-tRNA}$  with and without the addition of  $\text{BaCl}_2$  shows that, while the dependence of the  $\bar{R}$ -values on the concentration is lessened by the addition of 0.05 M  $\text{BaCl}_2$ , the values obtained by extrapolation to zero concentration are almost identical.

In Table 2 the ions used and their atomic weights are given, as well as the radii of gyration obtained after slit-correction and extrapolation to zero concentration (see Fig. 5). For comparison the  $R$ -value which we found in our first investigations [18, 19] of  $\text{tRNA}^{\text{Phe}}$  using a mixture of  $\text{K}^+$  and  $\text{Mg}^{++}$  ions is also given.

The  $\text{tRNA}^{\text{Phe}}$  consists of 76 nucleotides. If one assumes that one univalent cation belongs to the phosphate group of each nucleotide and one more ion to the phosphate group localized at the 5' end of the  $\text{tRNA}$  chain, 77 univalent ions would belong to each  $\text{tRNA}$  molecule. Assuming further that these ions belong to the molecule, the amounts of weight given

in Table 2 are to be added to the molecular weight of the  $\text{tRNA}^{\text{Phe}}$  of 24890 calculated from the sequence. It seems that the radius of gyration increases not with the equivalent weights of the cations, but with their atomic weights since the  $R$ -values obtained with  $\text{Cs}^+$  and  $\text{Ba}^{++}$  ions are nearly identical, as are also the atomic weights of the two ions.

In Fig. 6 the slit-corrected values of the radii of gyration which we obtained are plotted against the atomic weight of the cations used. The  $R$ -value obtained with  $\text{Cs}^+$  or  $\text{Ba}^{++}$  as counterion is about 10% higher than that obtained with  $\text{Li}^+$ .

In order to estimate whether this increase in the  $R$ -value is plausible, we carried out the following rough calculation. The  $\text{tRNA}^{\text{Phe}}$  molecule was approximated by a cylinder of axial ratio 1:2.5, and it was assumed that, for example, 77 cesium ions per  $\text{tRNA}^{\text{Phe}}$  molecule were uniformly distributed in a shell of 2 Å thickness (the round value of the ionic radius) around this cylinder. From this rough cal-

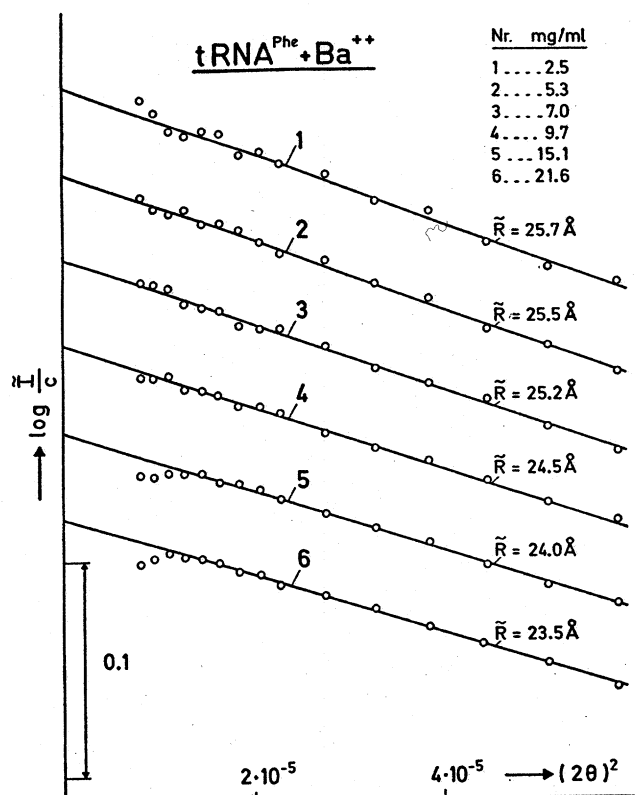


Fig. 3. Guinier plots of the scattering curves of  $Ba\text{-tRNA}^{\text{Phe}}$  in 0.05 M Tris-buffer, pH 7.5. The concentrations of  $\text{tRNA}^{\text{Phe}}$  and the apparent radii of gyration are also given in Table 1

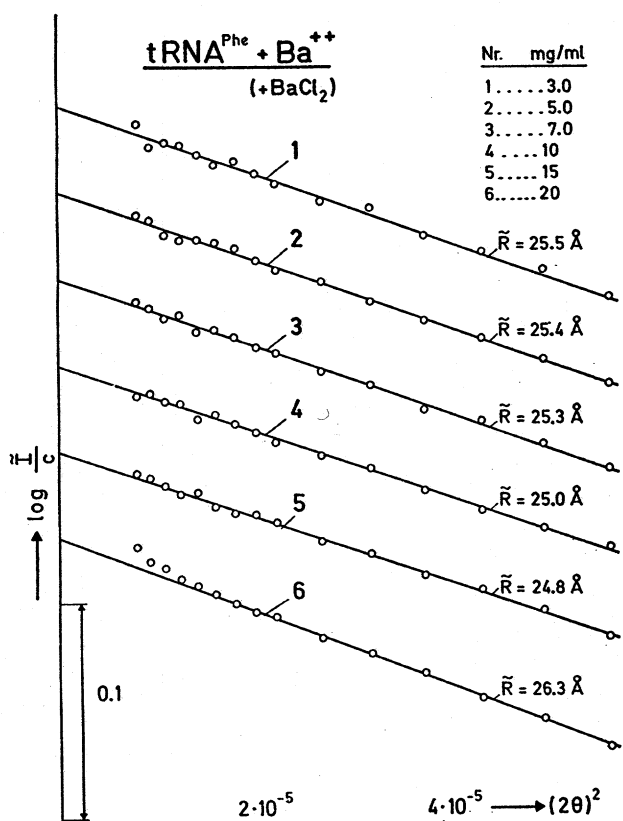


Fig. 4. Guinier plots of the scattering curves of  $Ba\text{-tRNA}^{\text{Phe}}$  in 0.05 M Tris-buffer, pH 7.5, additional 0.05 M  $\text{BaCl}_2$ . The concentrations of  $\text{tRNA}^{\text{Phe}}$  and the apparent radii of gyration are also given in Table 1

Table 2. Calculated molecular weights  $M$  and experimentally found radii of gyration  $R$  of  $\text{tRNA}^{\text{Phe}}$  (yeast) with various counter ions

1	2	3	4
Ion	Atomic weight of the ions	$M(\text{tRNA}) +$ weight of the counterions	$R$
			Å
$\text{Li}^+$	6.9	$24890 + 534 = 25424$	23.6
$\text{Cs}^+$	133	$24890 + 10230 = 35120$	26.2
$\text{Ba}^{++}$	137	$24890 + 5286 = 30176$	26.3
$\text{K}^+$	39	$24890 + 3011 = 27901$	
$\text{Mg}^{++}$	24	$24890 + 935 = 25825$	
$\text{Mg}^{++} + \text{K}^+$	24-39	(25800-27900)	24.4

culatation an increase in the  $R$ -value of 7% results from the transition from  $\text{Li-tRNA}$  to  $\text{Cs-tRNA}$ .

#### DISCUSSION

The present work shows clearly that the influence of the solvent, and especially that of the ions, must

in no case be disregarded when polyelectrolytes (in the present instance  $\text{tRNA}$ ) are investigated in solution. The work of Luzzati [8] and Bram [9] also clearly shows the great influence of the ions on the cross-section of DNA molecules. Eisenberg and Cohen [10] likewise indicate that very exact measurements are necessary in order to get a better

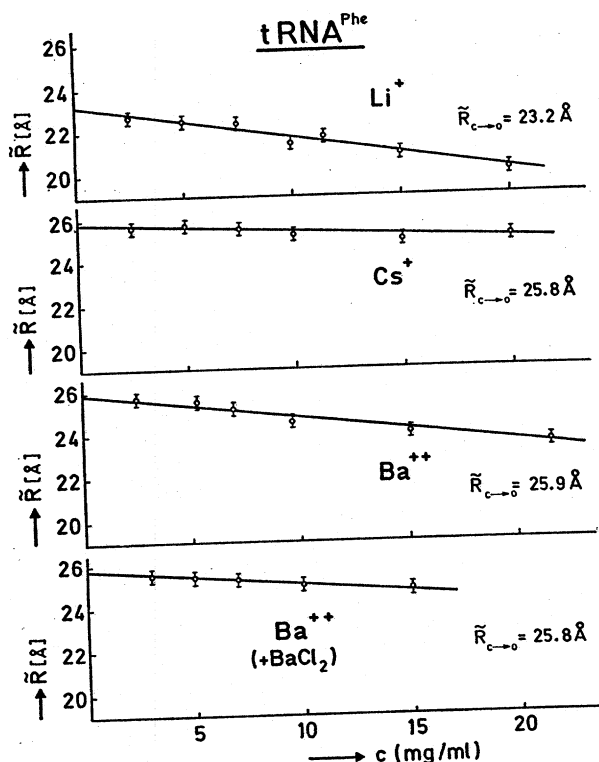


Fig. 5. Apparent radii of gyration  $\bar{R}$  of  $tRNA^{Phe}$ , summarized in Table 1, plotted against the concentration  $c$  of  $tRNA^{Phe}$  by using the ions given above and the buffers given in the legends of the Figs. 1–4. [One  $R$ -value (in Table 1 in brackets) which is by some reason affected with a greater error is not used in this plot]

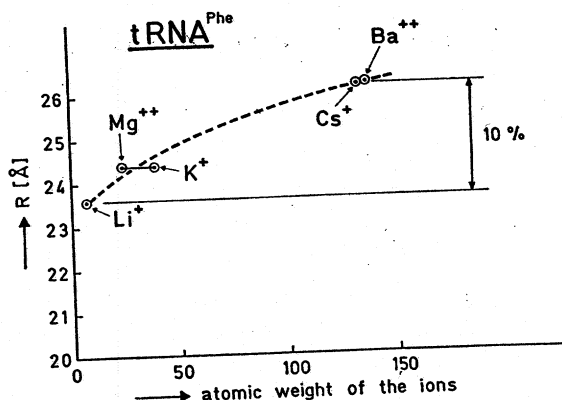


Fig. 6. Slit-corrected radii of gyration  $R$  of  $tRNA^{Phe}$  plotted against the atomic weight of the counterions used

overall view of the difficult problem of the ion and water shell.

The purpose of the work presented here was to determine the change in the radius of gyration of  $tRNA^{Phe}$  under the influence of various cations. The

influence of the ions was considerable; the value of  $R$  was, for instance, 23.6 Å with  $Li^+$  ions and 26.2 Å with  $Cs^+$  ions. This increase in the  $R$ -value lies roughly in the magnitude to be expected if one assumes that one univalent ion belongs to each phosphate group. This assumption is also usually made when experimental data are compared with the data calculated from the atomic coordinates of a proposed model. That this can be regarded only as a rough approximation is shown by the fact that the value of the radius of gyration is not dependent on the equivalent weight but on the atomic weight of the cation (see Table 2). If it were dependent on the equivalent weight a smaller  $R$ -value would be expected for  $Ba^{++}$ - $tRNA$  than for  $Cs^+$ - $tRNA$ . In point of fact, however, we find almost the same  $R$ -value for  $Cs^+$ - and  $Ba^{++}$ - $tRNA$ .

This result clearly shows that hydration must also be an essential factor. Satisfactory theoretical treatment of the structure of the water shell has not yet been successful even with simple ions [7], and, obviously, more complex polyelectrolytes will present even greater problems.

Finally it should be noted that the type of solvent used naturally influences not only the radius of gyration and molecular weight but also the entire scattering curve, especially the tail-end. It is thus also difficult to carry out detailed determinations of the conformation of the  $tRNA$  molecule by means of comparison of the experimental curves with scattering curves calculated from the atomic coordinates. We shall at present have to content ourselves with obtaining an overall shape as long as the influence of the counterions and hydration cannot be precisely accounted for.

This research has been financed in part by a grant made by the United States Department of Agriculture under P.L. 480. This work was also supported by research grants from the Österreichische Forschungsrat. We (I. P. and O. K.) wish to thank Miss M. Schuster and Miss B. Müller for experimental help.

## REFERENCES

- Holley, R. W., Apgar, J., Everett, G. A., Madison, J. T., Marquisee, M., Merrill, S. H., Penswick, J. R., and Zamir, A., *Science*, 147 (1965) 1462.
- Cramer, F., Doepner, H., Haar, F. v. d., Schlimme, E., and Seidel, H., *Proc. Nat. Acad. Sci. U. S. A.* 61 (1968) 1384.
- Ninio, J., Favre, A., and Yaniv, M., *Nature (London)*, 223 (1969) 1333.
- Connors, P. G., Labanauskus, M., and Beeman, W. W., *Science*, 166 (1969) 1528.
- Levitt, M., *Nature (London)*, 224 (1969) 759.
- Krigbaum, W. R., and Kügler, F. R., *Biochemistry*, 9 (1970) 1216.
- Oosawa, F., *Biopolymers*, 9 (1970) 677.
- Luzzati, V., Masson, F., Mathis, A., and Saludjian, P., *Biopolymers*, 5 (1967) 491.

9. Bram, S., Ph. D. Thesis, University of Wisconsin 1968.
10. Eisenberg, H., and Cohen, G., *J. Mol. Biol.* 37 (1968) 355.
11. Kratky, O., *Z. Electrochem.* 58 (1954) 36; 62 (1958) 66.
12. Kratky, C., and Kratky, O., *Z. Instrum.* 72 (1964) 302.
13. Leopold, H., *Elektronik*, 10 (1965) 359.
14. Zipper, H., *Acta Phys. Austriaca*, 30 (1969) 143.
15. Guinier, A., and Fournet, G., *J. Phys. Radium*, 8 (1947) 345.
16. Heine, S., and Roppert, J., *Acta Phys. Austriaca*, 15 (1962) 148.
17. Guinier, A., and Fournet, G., in *Small Angle Scattering of X-Rays*, Wiley, London 1955.
18. Pilz, I., Kratky, O., Cramer, F., Haar, F. v. d., and Schlimme, E., *Eur. J. Biochem.* 15 (1970) 401.
19. Kratky, O., Pilz, I., Cramer, F., Haar, F. v. d., and Schlimme, E., *Monatsh. Chem.* 100 (1969) 748.

I. Pilz and O. Kratky  
 Institut für Physikalische Chemie der Universität  
 Heinrichstraße 28, A-8010 Graz, Austria

F. von der Haar and F. Cramer  
 Max-Planck-Institut für Experimentelle Medizin  
 Abteilung Chemie  
 BRD-3400 Göttingen, Hermann Rein-Straße 3  
 German Federal Republic